European Lipoprotein Club

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Meeting report

European Lipoprotein Club: Report of the 30th ELC annual conference, Tutzing, 3–6 September 2007

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1. Introduction

The 30th meeting of the European Lipoprotein Club was held from 3 to 6 September at the Evangelische Akademie in Tutzing Germany and attended by some 120 participants from all over Europe and the United States. On Monday night, after the welcome by the ELC secretary, Marten Hofker, two state of the art lectures were presented by, respectively, Klaus Lindtpaintner (Basel, Switzerland) and Göran Hansson (Stockholm, Sweden). Klaus Lindtpaintner spoke on "Biomarkers and personalized medicine: hype, hope and importance of being earnest". Göran Hansson's presentation was entitled: "Grease or heat? Liposuction or Aspirin? Thoughts on the Pathology and Treatment of Atherosclerosis".

On Tuesday, the first session focused on vascular biology and atheroslerosis and was introduced by Ira Tabas (New York, USA). His presentation was entitled: "From atherosclerosis to innate immunity; lessons from studying how macrophages die". The second session revolved around lipid transport and was introduced by Daniel Rader (Philadelphia, USA), with a presentation entitled: "Molecular regulation of reverse cholesterol transport". On Wednesday, the third session focused on diagnostic and therapeutic targets. Lawrence Chan (Houston, USA) presented an invited lecture on the advances in gene therapy for familial hypercholesterolemia and diabetes. The fourth session on gene regulation was introduced by Richard Deckelbaum (New York, USA) and he presented a lecture entitled: "Post-transcriptional regulation of SREBP by three interdependent but distinct mechanisms". The fifth session on metabolic syndrome was introduced by Mark McCarthy (Oxford, UK) on genetic and genomic biomarkers for type 2 diabetes. The final "Varia" session on Thursday, consisted exclusively of speakers invited on the basis of their submitted abstracts.

To promote participation by junior researchers, two types of awards were presented to two young scientists. The young investigator award was presented to Oliver Bruns (Hamburg, Germany) for his presentation entitled: "Non-invasive in vivo imaging of recombinant TRL labeled with nanocrystals". The poster award was presented to Philip Gordts (Leuven, Belgium) for his poster entitled: "Inactivation of the NPXYXXL domain and downstream motifs of LRP1 show a differential increase in atherosclerosis". The winner of the poster prize was also given the opportunity to orally present his poster in the "Varia" session.

2. State of the art lecture

Chaired by Marten Hofker (Groningen, The Netherlands).

The scientific part of this year's meeting was opened by two state of the art lectures that provided future perspectives from both industry and academia. *Klaus Lindtpaintner* (Roche, Basel) opened with his presentation that was adequately entitled "Biomarkers and Personalized Medicine: Hype, Hope and Importance of Being Earnest". He shared his view of the hypes around personalized medicine, where everybody is expecting a sudden revolution based on the novel technologies in biomedical research. He pointed out, however, that in the field of cardiovascular diseases it is already customary to carry out careful diagnosis and therapy based on biomarkers. In particular, measurements of lipoproteins and the interpretation of the lipoprotein profiles are being done with high accuracy. There are excellent algorithms, such as Procam, to further aid the interpretation of the data. He doubted if it would be possible soon to identify novel biomarkers that would markedly improve our ability to predict disease susceptibility or to guide treatment. Although striking improvements are rare, they sometimes do occur.

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One of the best examples of success of genetic stratification of patients to select responders to a particular treatment is the use of the anticancer drug Iressa in certain forms of small cell lung cancer. This treatment is very effective in carriers of a susceptible Epidermal Growth Factor Receptor (EGFR) allele. Unfortunately, examples such as this are rare. One should not expect genomic approaches to easily lead to the discovery of genetic variants or biomarkers showing similar discriminatory power. Dr. Lindpaintner was earnest in stating that genetics and other sources of potential new biomarkers simply did not deliver the tools for improving prediction yet, and also the effect sizes observed with genetic risk alleles prohibit current application in diagnosis. Nevertheless, he remained optimistic that the current research would gradually improve this situation and that serendipitous findings may occur, reminiscent of Iressa, so that the goal of personalized medicine becomes reality.

The lecture from *Göran Hanson* (Stockholm, Sweden) was entitled: "Grease or heat? Liposuction or Aspirin? Thoughts on the Pathology and Treatment of Atherosclerosis". He pointed out that much of the insight into the mechanisms of atherosclerosis has been driven by research in the area of lipid metabolism and that lipid lowering has been and still is very important in the battle against cardiovascular diseases. However, he also explained that lipid lowering only has modest effects on the hard end points, such as CVD death. When studying the pathogenesis of atherosclerosis, it becomes clear that inflammation plays a crucial role. Hence, for defining novel therapeutic targets, it is very important to understand the role of the immune system in atherosclerosis.

A significant amount of immune reactivity can be observed in the plaque as evidenced by the presence of macrophages and T-cells, MHC molecules, and inflammatory mediators such as cytokines. Moreover, LDL, one of the major atherogenic lipoproteins, can undergo immunogenic modifications and yield phospholipid fragments (e.g. phosphorylcholine and PAPC) and modified APOB, which in turn can be recognized by the immune system. One of the major goals is to identify the "bottleneck" factors that drive atherogenesis. One of these potential bottlenecks could be the tumor necrosis factor (TNF) gene family. Interestingly, TNF is linked to metabolic processes and is well known to inhibit lipoprotein lipase (LPL). Recently, however, the role of another TNF gene family member, LIGHT, has attracted considerable attention. This gene is mainly expressed in T-cells and dendritic cells. Remarkably, overexpression of LIGHT on T-cells in mice leads to hyperlipidemia. This effect could be reversed when blocking the lymphotoxin-beta receptor, the main receptor for LIGHT. Interestingly, also other members of the TNF family, such as OX40L and CD137 play an important role in T-cells and have marked effects on atherogenesis. These studies have disclosed an important relationship between T-cell genes, metabolism and cardiovascular disease and open up exciting opportunities for the development of novel therapies.

3. Session I

Vascular biology and atherosclerosis: chaired by Ko Willems van Dijk (Leiden, The Netherlands) and Ken Lindstedt (Helsinki, Finland).

The vascular biology and atherosclerosis session was opened by *Ira Tabas* (New York, USA) on the lessons learnt from dying macrophages in atherosclerosis and innate immunity. The formation of a necrotic core, consisting of dead and dying macrophages in an atherosclerotic lesion constitutes the main risk factor for lesion rupture. Such a lesion has also been termed the vulnerable plaque. Ira Tabas started off by warning the audience about the complexity of the processes that are involved in the formation of the necrotic core. Many hits are required for the induction of macrophage apoptosis and any single hit is not sufficient as one would expect for such a potentially dangerous process. He identified at least four different players in macrophage apoptosis: (1) endoplasmic reticulum (ER) stress via the unfolded protein response (UPR), which is induced, for example, by oxidized phospholipids; (2) the scavenger receptor A (SRA), which is activated by modified lipoproteins; (3) the toll like receptor 4, which is activated by SRA ligands, saturated fatty acids, and other substances in lesions; and (4) a rise in cytosolic calcium levels.

Both the SRA and TLR4 are pattern recognition receptors. Activation of TLR4 results in the induction of both a cell survival pathway via the TRIF-TRAM-IRF-3-IFNb branch of TLR4 signaling and apoptosis via the Myd88-Mal branch. In the presence of "classic" TLR4 activation, such as via LPS, the cell survival pathway is dominant over the apoptosis pathway. However, in the presence of SRA ligands, such as modified lipoproteins that are present in the lesion, the cell survival pathway is selectively shut down and the cell will go into apoptosis—all in the setting of ER stress.

The interaction between pattern recognition receptors such as the SRA and TLR4 (and other previously described combinations) could be a means to provide an innate host defense against organisms that hide inside living macrophages. Intriguingly, this evolutionary conserved mechanism wreaks havoc in the atherosclerotic plaque and renders it vulnerable to rupture.

Ernst Malle (Graz, Austria) showed that the soluble part of the receptor for advanced glycation end products (sRAGE) binds with high affinity to hypochlorous acid-modified LDL (HOCl-LDL), and thereby effectively inhibits uptake and accumulation of HOCl-LDL by macrophages. sRAGE also form a co-precipitable complex with HOCl-LDL in serum. sRAGE was found to specifically inhibit the interaction between HOCl-LDL and the scavenger receptor CD36. Finally, Malle proposed that sRAGE, by being a physiological antagonist for CD36-mediated accumulation of cholesterol in phagocytes, may act as a sink for HOCl-LDL in atherosclerotic lesions.

The study by *Cinzia Parolini* (Milan, Italy) evaluated, in vivo, the minimal dose of apoA-IMilano phospholipid complex (ETC-216) able to induce atherosclerosis regression in

a rabbit model of lipid-rich plaques. Thirty-six rabbits underwent perivascular injury at both carotids, followed by a 1.5% cholesterol diet. After 90 days, rabbits were randomly divided into six groups and treated five times with vehicle or different doses of ETC-216, once every four days. Carotid plaque changes were evaluated in vivo by IVUS and MRI. The minimal effective dose needed for regression of atherosclerosis (−1.56%) was found to be 20 mg/kg and the best result (−6.83%) was obtained with the maximal dose of ETC-216 used. A significant regression was achieved with only two administrations of the highest dose. The author concluded that repeated infusions of low doses of ETC-216 effectively affected atherosclerosis in an animal model.

Ko Willems van Dijk (Leiden, The Netherlands) presented data on a role for maternal genotype and associated hypercholesterolemia on the developing vascular systems in heterozygous apoE-deficient mice. Carotid artery plaque formation was induced in adult mice by a constrictive perivascular collar placement. The authors found that maternal apoE-deficiency affected the carotid endothelial function in fetuses, but found no signs of fatty streak formation in pre- or postnatal animals. However, collar-induced carotid lesion development was highly increased in adult offspring of maternal apoE-deficiency compared to offspring of maternal wild type. The author concluded that maternal apoE-deficiency not only affects the endothelium of fetal arteries but also increases the susceptibility for development of collar-induced atherosclerosis in adult life, by epigenetic mechanisms.

4. Session II

Lipid transport: chaired by Franco Bernini (Parma, Italy) and Joerg Heeren (Hamburg, Germany).

The lipid transport session was opened with an invited lecture given by *Daniel Rader* (Philadelphia, USA) on the molecular regulation of reverse cholesterol transport (RCT) in vivo. He first reported results obtained modulating the host mice specifically using a vector based on adeno-associated virus serotype 8 (AAV8) to evaluate the effect of CETP expression in mice (which naturally lack CETP) on macrophage RCT. The experiment included mice that lacked the low-density lipoprotein receptor or the scavenger receptor class B, type I (SR-BI). He demonstrated that CETP expression promotes macrophage RCT in mice, that this effect is dependent on the low-density lipoprotein receptor, and that CETP expression restores to normal the impaired RCT in mice deficient in SR-BI. Based on these findings he raised the possibility that the CETP inhibitors may have beneficial effects for some people and not for other based on the expression of LDL receptors. Dr. Rader also investigated the still unknown contributions of macrophage ATP-binding cassette transporter A1 (ABCA1), SR-BI, and ABCG1 in mediating RCT from macrophages in vivo. To achieve this aim, pharmacological means, such as LXR agonists, were used combined with the injection in host mice of genetically manipulated primary macrophages. He reported that macrophages lacking ABCA1 had a significant reduction in macrophage RCT in vivo, demonstrating the importance of ABCA1 in promoting macrophage RCT, however, substantial residual RCT existed in the absence of macrophage ABCA1. Using macrophages deficient in SR-BI expression, he showed that macrophage SR-BI does not contribute to macrophage RCT in vivo. To investigate whether ABCG1 was involved in macrophage RCT, he used ABCG1-overexpressing, -knockdown, and -knockout macrophages showing that increased macrophage ABCG1 expression significantly promoted while knockdown or knockout of macrophage ABCG1 expression significantly reduced macrophage RCT in vivo. The additive effect of both ABCA1 and ABCG1 in promoting macrophage RCT in vivo was also demonstrated. The risk of atherosclerosis is inversely associated with plasma levels of high-density lipoprotein cholesterol (HDL-C). However, Dr. Rader underlined that HDL metabolism is incompletely understood, and there are few effective approaches to modulate HDL-C levels. He reported that inhibition in the liver of the classical pro-protein convertases (PCs), decreases plasma HDL-C levels. This metabolic effect of hepatic PCs is critically dependent on expression of endothelial lipase (EL), an enzyme that directly hydrolyzes HDL phospholipids and promotes its catabolism. Hepatic PCs reduce EL function through direct inactivating cleavage of EL as well as through activating cleavage of angiopoietin-like protein 3 (ANGPTL3), an endogenous inhibitor of EL. Inhibition of hepatic PCs results in increased EL activity and reduced HDL-C as well as impaired RCT in vivo. Therefore, the hepatic PC-ANGPTL3-EL-HDL pathway could be a novel mechanism controlling HDL metabolism and cholesterol homeostasis.

Carlos Vrins (Amsterdam, The Netherlands) discussed the effect of PPAR-delta activation on the intestinal cholesterol efflux pathway. Previous studies have shown that activation of PPAR-delta is associated with an increase of fecal sterol secretion, which could only partially be explained by reduced cholesterol absorption. This study was aimed to investigate whether this activation not only affects intestinal absorption but also secretion. The author reported that treatment of FVB wild type mice with the PPAR-delta agonist GW610742X did induce the intestinal cholesterol efflux without affecting total serum cholesterol and the hepatobiliary cholesterol secretion. The reduction in intestinal NPC1L1 gene expression indicated the expected change in intestinal cholesterol absorption as reported earlier. Possible involvement of NPC1L1 in intestinal cholesterol efflux was tested with the inhibitor ezetimibe. Feeding mice with ezetimibe did not affect intestinal cholesterol secretion. Based on these results, it was concluded that the previously observed increase in neutral sterol secretion upon PPAR-delta activation can be explained in part by the increased direct intestinal cholesterol secretion.

Zhao-Yan Jiang (Stockolm, Sweden) discussed the possible contribution of several hepatic genes to biliary cholesterol super-saturation, a prerequisite for cholesterol gallstone formation in humans. Comparing age and BMI matched groups

of gallstone patients (GS) and gallstone free patients (GSF), it was found that in GS, gallbladder bile was more saturated with cholesterol than in GSF, due to increased molar % of cholesterol. The expression of ABCG5, ABCG8, and of LXR-alpha was increased in GS patients. In addition, the mRNA and protein of SR-BI were also increased in GS patients as compared to GSF subjects. The increased expression of the canalicular cholesterol transporters ABCG5 and ABCG8 may account for super-saturation of the bile with cholesterol in GS patients and the excessive biliary cholesterol may originate from the uptake of plasma HDL cholesterol via increased expression of SR-BI.

Ryan E. Temel (Winston-Salem, USA), reported on the role of hepatic Niemann-Pick C1-Like 1 (NPC1L1) in biliary cholesterol concentration. Humans highly express NPC1L1 in the liver but the exact function of hepatic NPC1L1 is unknown. They recently described that human NPC1L1 expression in hepatoma cells facilitates free cholesterol uptake from the culture medium. Creating transgenic mice overexpressing NPC1L1 they tested the hypothesis that hepatic NPC1L1 allows the retention of biliary cholesterol by hepatocytes and that ezetimibe disrupts the hepatic function of NPC1L1. The overexpression of NPC1L1 resulted in a 10- to 20-fold decrease in biliary cholesterol concentration associated with a 30%–60% increase in plasma cholesterol. Biliary and plasma cholesterol levels were almost normalized with ezetimibe treatment suggesting that in humans, ezetimibe may reduce plasma cholesterol by inhibiting NPC1L1 functions in both intestine and liver, and that hepatic NPC1L1 may protect the body from excessive biliary loss of cholesterol.

The second invited speaker of this session *Laurent Lagrost* (Dijon, France) described the pleiotropic effects of plasma phospholipid transfer protein (PLTP). PLTP belongs to the lipid transfer/lipopolysaccharide binding protein family that includes the cholesteryl ester transfer protein (CETP), the bactericidal permeability increasing protein and the lipopolysaccharide-binding protein. Dr. Lagrost showed that PLTP can act in several distinct metabolic processes such as the molecular transfer of phospholipids and alpha tocopherol but also lipopolysaccharides suggesting that PLTP might be involved both in lipoprotein metabolism and in antimicrobial defence. The potential role of PLTP in the development of atherosclerosis has not been fully elucidated. While systemic PLTP activity correlate positively with the risk of cardiovascular disease, an atheroprotective role of macrophage-derived PLTP was observed in LDL receptor deficient and apolipoprotein E deficient mice. In diabetic patients, who have increased plasma PLTP activity, the lipoprotein distribution of alpha tocopherol is dependent on PLTP activity. To circumvent the problem that mice do not express CETP, Dr. Lagrost performed studies with PLTP-transgenic rabbits which are characterized by a more human-like lipoprotein metabolism. There was no direct correlation between the concentration of alpha tocopherol and lesion area in these animals. However, most interestingly, compared to the control groups PLTP-transgenic rabbits have (a) more atherosclerotic lesions, (b) increased levels of LDL cholesterol and (c) reduced alpha tocopherol levels demonstrating the important role of PLTP for lipoprotein metabolism and the development of atherosclerosis.

Lars Bo Nielsen (Copenhagen, Denmark) described in his presentation the effect of apolipoprotein M (apoM) on HDL metabolism and atherosclerosis. ApoM is a novel apolipoprotein mainly present in high-density lipoprotein (HDL). It belongs to the lipocalin protein superfamily and is secreted without cleavage of its hydrophobic signal peptide, which probably anchors apoM in the phospholipid layer of plasma lipoproteins. Dr. Nielsen showed that the concentration of HDL is increased in mice which express the human apoM-transgene, while apoM-deficient mice have decreased HDL levels. Detailed studies analyzing the lipoprotein composition did not reveal differences in the size and charge of HDL between the different genotypes analyzed. However, the conversion of HDL particles mediated by LCAT-dependent and independent mechanisms were influenced by the presence of apoM. Moreover, apoM expression protects against the development of atherosclerosis on the LDL receptor deficient background which might be explained by (a) the modulation of plasma HDL particles, (b) by apoM-facilitated cholesterol efflux or (c) by the anti-oxidative effect of apoM.

Stefan K. Nilsson (Umeå, Sweden) provided new insights in the triacylglycerol (TG)-lowering effect of apolipoprotein A-V (apoAV). ApoAV has been shown to modulate the lipolytic activity of lipoprotein lipase. However, more than 99% of apoAV is associated with the liver and therefore the author proposed that apoAV might influence the internalization of TG-rich lipoproteins by mediating the interaction of lipoproteins with their respective receptors. By surface plasmon resonance studies, he showed that both lipid-free and lipidated apoAV interacts with LRP1 and SorLA, both members of the LDL receptor family, as well as with the Sortilin receptor of the Sortilin gene family. Furthermore, the binding of chylomicrons to these receptors was enhanced in the presence of apoAV. In cellular studies using HEK293 cells transfected with SorLA, and with cells transfected with Sortilin, he showed that lipid-associated apoAV is internalized via receptor-mediated endocytosis. The author concluded that apoAV exerts its TG-lowering effect by receptor mediated lipoprotein clearance.

Willeke de Haan (Leiden, The Netherlands) identified the minimal domain of apolipoprotein CI (apoCI) that specifically inhibits the activity of CETP. ApoCI is present on TG-rich lipoproteins and HDL and has several roles in lipoprotein metabolism, such as the inhibition of lipoprotein lipase, inhibition of the apoE-mediated hepatic remnant clearance and inhibition of CETP. To identify a novel HDL-raising and therefore potential anti-atherogenic drug, the author created different apoC-I peptides and analyzed the potential of these molecules to inhibit CETP and LPL activity. Whereas apoC-I N-terminal fragments showed only negligible CETP inhibition, a C-terminal peptide (32–57) dose-dependently

inhibited CETP activity. This CETP inhibitory effect was mainly dependent on positively charged residues. Since LPL activity was not influenced by the apoCI 32–57 peptide, the author concluded that this fragment might be an interesting novel 'drugable' CETP inhibitor.

Lucia Rohrer (Zurich, Switzerland), investigated how high density lipoprotein HDL and its major protein constituent apolipoprotein apoA-I are transported from the blood stream into the vascular wall to exert their anti-atherogenic properties within the arterial wall. They previously showed that ABCA1 mediates apoA-I transcytosis in endothelial cells. Here the author specifically looked at the interaction of HDL with the endothelial cells. It was found that endothelial cells bound both I^{125}-apoAI and I^{125}-HDL with high affinity and in a saturable manner and that binding and cell association of HDL was only competed by excess of HDL and not by apoA-I or albumin. Through biotinylation experiments, it was also demonstrated that endothelial cells internalize labeled HDL and that only a minor amount of the internalized HDL was degraded. Modulating ABCG1 and SR-BI with RNA interference it was demonstrated that HDL transport was reduced indicating that endothelial cells transcytose HDL in an ABCG1 and/or SR-BI dependent process.

5. Session III

Diagnostic and therapeutic targets: chaired by Dilys Freeman (Glasgow, UK) and Florian Kronenberg (Innsbruck, Austria).

Lawrence Chan (Houston, Texas, USA) gave an overview on the use of gene therapy to treat familial hypercholesterolaemia (FH) and diabetes. In an LDL receptor knockout mouse model of FH, gene transfer of the LDL receptor resulted in a marked reduction in plasma cholesterol, increased LDL turnover, reduced atherosclerosis and longer survival. Similar studies in heterozygous FH rhesus monkeys using the LDL receptor gene in an helper-dependent vector injected into the hepatic artery were somewhat less successful with a requirement for high gene doses and a short duration of response. The duration of response was improved by using a balloon catheter based delivery system to occlude hepatic venous drainage. The second topic looked at type 1 and 2 diabetes which feature pancreatic beta cell dysfunction. Gene transfer of pro-insulin or modified insulin with a glycaemia-regulated promoter has not worked well in experimental animals since the physiological regulation of insulin secretion is not replicated. An alternative approach is to induce beta cell formation in the liver by the delivery of developmental transcription factors to the liver. Transfer of PDX-1 into a streptozotocin-induced mouse model of diabetes, although increasing plasma insulin and reducing plasma glucose resulted in severe fulminant hepatitis due to the concomitant induction of exocrine pancreatic cells. Transfer of a transcription factor further downstream in the developmental pathway of endocrine beta cells, NGN3, along with the gene for a beta cell stimulating hormone, betacellulin, restored insulin levels and glucose tolerance in the diabetic mice. Furthermore, plasma insulin levels were downregulated in response to fasting. One approach to treating insulin resistance is to reverse obesity. Chan and colleagues have interrupted the capillary blood supply to adipose tissue by targeted induction of apoptosis. Blood vessels supplying particular tissues are unique and express difference surface molecules. Phage display was used to identify a peptide motif that acts as a marker of the vasculature of adipose tissue. A pro-apoptotic peptide was then targeted to this marker resulting in fat ablation. In mice, molecular fat ablation does not result in accumulation of fat in other tissues and metabolic abnormalities were corrected, possibly because molecular ablation targets both subcutaneous and visceral fat. It will be fascinating to discover whether peptide-mediated fat ablation will work in humans.

Patrick Rensen (Leiden, The Netherlands) reported their investigations on the HDL-raising effects of niacin. They used female APOE*3-Leiden transgenic mice with the human CETP transgene under control of its natural flanking regions and fed them with a Western-type diet with or without niacin. Niacin dose-dependently decreased plasma total cholesterol and triglycerides and increased HDL-C and apoAI. Interestingly, APOE*3-Leiden mice that do not express CETP showed no HDL-increasing effect of niacin. The authors further showed that niacin dose-dependently decreased plasma CETP mass and activity. These experiments clearly demonstrated that the HDL-increasing effect of niacin is caused by a decreased cholesteryl ester transfer from HDL to (V)LDL.

Ivan Tancevski (Innsbruck, Austria) presented their results on the novel liver-selective thyromimetic T-0681 which displays strong hypolipidemic properties without cardiotoxic effects. The authors used BALB/c mice and New Zealand White rabbits fed a high-cholesterol diet. They showed with these two models that T-0681 decreases plasma cholesterol and triglycerides levels by a pronounced increase in the hepatic expression of scavenger receptor-BI as well as the LDL receptor without changes in plasma CETP-activity. Additionally, T-0681 was shown to promote reverse cholesterol transport from macrophages to faeces in vivo. In cholesterol fed rabbits, treatment with T-0681 led to a 60%-decrease of atherosclerotic lesion area. These data suggest that liver-selective thyromimetics may prove useful therapeutic agents against the development of atherosclerosis in humans.

Lena Persson (Huddinge, Sweden) investigated the role of PCSK9 in the hormonal regulation of hepatic LDL receptors (LDLRs). The experiments showed that cholesterol-feeding reduced LDLR mRNA whereas LDLR protein was induced; concomitantly PCSK9 mRNA was reduced. Similar effects were observed for glucagon. However, estrogen treatment induced LDLR mRNA and LDLR protein; concomitantly PCSK9 mRNA was reduced. Other hormones such as thyroid hormone, growth hormone, insulin and IGF-1 were also investigated. PCSK9 showed a pronounced and highly significant regulation by a broad set of hormones previously

established to modulate hepatic LDL receptor expression. These findings suggest that PCSK9 is important in the hormonal regulation of hepatic LDLRs.

6. Session IV

Gene Regulation: chaired by Gertrud Schuster (Davis, USA) and Paolo Parini (Stockholm, Sweden).

Richard Deckelbaum (New York, USA) gave an overview on their recent findings on the "post-transcriptional regulation of SREBP by three independent but distinct mechanisms". Sterol regulatory element binding protein (SREBP) mediates the functions of fatty acids and cholesterol by binding to sterol regulatory elements (SRE) present in the promoter of genes involved in various aspects of lipid and cholesterol homeostasis such as plasma lipid levels, intracellular lipid content, and membrane physiology. SREBP precursor protein (pSREBP) is embedded in the ER and is known to be activated by sterol depletion due to proteolysis by interacting with another ER membrane protein SREBP cleavage-activating protein (SCAP); however, the question of whether the conversion of pSREBP to mature SREBP (mSREBP) is a single essential or multiple control pathway still remains.

In previous studies Richard Deckelbaum and colleagues (Tilla S. Worgall and Rebecca Juliano) showed that fatty acids regulate SRE-mediated gene transcription by decreasing mSREBP in the absence of cholesterol as well as synergistically with cholesterol. In addition, fatty acids can affect SRE-mediated gene transcription by two independent mechanisms. (1) Fatty acids can induce sphingomyelin hydrolysis, which can result in the intracellular displacement of membrane cholesterol. (2) sphingomyelin hydrolysis also results in the generation of ceramide, which can decrease levels of transcriptionally active mSREBP and SRE-mediated gene transcription. In addition, they unraveled with fluorescence tagged proteins that cholesterol, ceramide, and unsaturated fatty acids regulate SREBP processing at different points. Whereas cholesterol inhibits the translocation of SREBP and SCAP into the nucleus and Golgi apparatus, but not general vesicular trafficking, ceramide disrupts all vesicular trafficking of SREBP and SCAP from the ER to the Golgi apparatus. Linoleic acid prevents their migration into the nucleus, from the ER and Golgi, but does not inhibit SCAP trafficking from the ER to the Golgi apparatus. In conclusion these data open the door for the development of new pathways and perhaps therapeutics agents interfering with the SREBP trafficking pathways and the regulation of genes involved in lipid and cholesterol homeostasis.

Harald Funke (Jena, Germany) reported on the interactions of monocytes and T-lymphocytes with the vessel wall from patients with familial hypercholesteremia (FH), leading to differences in intracellular lipoprotein metabolism. They found a considerable amount of genes that are up-regulated in T-lymphocytes in FH patients, suggesting an accelerated activation of these cells. Freshly isolated homozygous FH monocytes are characterized by increased distribution of scavenger receptors, like CD36, CD68, and low-density lipoprotein-related protein 1 (LRP-1) which is associated with elevated uptake of native as well as oxidized LDL. Although in patients with FH the circulating number of CD14+/CD16+ positive monocytes is decreased, there is an increase of CD29 and CD11c, which are typically expressed in dendritic cells.

Mohamed Amine Bouhel (Lille, France) presented on the ability of PPAR-gamma activation to prime human monocytes into alternative M2 macrophages with anti-inflammatory properties. Th1 cytokines promote the M1 pro-atherogenic activation, while Th2 cytokines attenuate macrophage-mediated inflammation leading to an "alternative" M2 macrophage phenotype. In the presence of IL-4, PPAR-gamma has the ability to prime primary human monocytes toward alternative M2 differentiation. When PPAR-gamma is stimulated, cell culture medium from M2 differentiated macrophages exerts more pronounced anti-inflammatory properties on M1 macrophages than the medium from un-stimulated M2 macrophages. PPAR-gamma activation did not affect the expression of M2 markers in resting and in M1 macrophages, suggesting that only native monocytes could be primed by PPAR-gamma to an enhanced M2 phenotype. Finally in humans, PPAR-gamma activation by pioglitazone administration significantly increased the expression of the M2 marker CD206 in blood monocytes. These data together suggest that PPAR-gamma may exert anti-inflammatory activities by promoting the differentiation of human monocytes toward a M2 phenotype.

The session was concluded with a contribution from *Ruth Frikke-Schmidt* (Copenhagen, Denmark) who presented on the predictive power of a functional promoter variant in the zinc finger protein 202 for severe atherosclerosis and ischemic heart disease (IHD). ZNF202 is a transcriptional repressor controlling promoter elements found in genes involved in vascular maintenance and lipid metabolism. The hypothesis tested was that a common variant, g.-660A > G, in ZNF202 promoter is functional, predicts severe atherosclerosis, and predicts ischemic heart disease. In vitro, ZNF202 g.-660G versus g.-660A was associated with a 60% reduction in transcriptional activity. In a cross-sectional study of 5467 individuals from the Danish general population, GG-versus AA-homozygosity predicted an odds ratio for severe atherosclerosis of 2.01. In 10,522 individuals from the Danish general population, The Copenhagen City Heart Study, including 1526 incident IHD events during 28 years of follow-up, GG-versus AA-homozygosity predicted a hazard ratio for IHD of 1.21(1.02–1.35). Finally in two independent case–control studies including, respectively, 943 and 1549 cases with IHD and 8996 controls, equivalent odds ratios for IHD were 1.29 (1.02–1.62) and 1.60 (1.34–1.92). In conclusion, homozygosity for a common functional promoter variant in the transcriptional repressor ZNF202 predicts severe atherosclerosis and an increased risk of IHD.

7. Session V

Metabolic syndrome: chaired by Fredrik Karpe (Oxford, UK) and Folkert Kuipers (Groningen, The Netherlands).

The session on the metabolic syndrome was initiated by *Mark McCarthy* (Oxford, UK) who reviewed the recent rapid progress in identifying novel genes for type 2 diabetes and obesity. The discovery of the genetic background to monogenic forms of diabetes has been addressed with conventional techniques through segregation analysis, whereas the common genetic variants underlying complex diseases have been elusive and often difficult to replicate. The problem has been very much described as an issue of power and recent developments of novel technological platforms together with large and aggregated patient cohorts (for example, the Wellcome Trust Case-Control Consortium) has made it possible to identify several novel targets, of which the obesity FTO gene is a good example. Not unexpectedly, such common genes show low odds ratio for disease when assessed properly. The next challenge is to search for less common genetic variants (allele frequencies of 1–5%), probably with higher odds ratio for disease; that gap is still to be filled for a more complete understanding of the genetic background to obesity and diabetes.

Børge Nordestgaard (Herlev, Denmark) described the power of non-fasting triglycerides as risk marker for myocardial infarction, ischemic heart disease and death in the Copenhagen City Heart study including more than 13,000 people followed for 26 years. Non-fasting triglycerides include remnant lipoproteins and the atherogenic properties of such lipoproteins were offered as an explanation for the strong positive relationship between triglycerides and heart disease.

Hans Dieplinger (Innsbruck, Austria) described the identification of afamin, a vitamin E-binding glycoprotein, as strongly related to the metabolic syndrome. This was achieved using a comparative proteomic approach and then searching for an association between afamin concentrations and parameters of the metabolic syndrome in the Bruneck study ($n = 825$). A causal relationship of unknown nature was suggested as mice with a transgenic overexpression of afamin show signs of metabolic dysregulation compatible with the metabolic syndrome.

Bert Groen (Amsterdam, The Netherlands) described investigations of the use of iminosugars in the regulation of features of the metabolic syndrome in mice. The iminosugar AMP-DNM upregulated CYP7A1 and drastically altered the bile composition. The mechanism for this effect appeared to be mediated through inhibition of FGF19, which otherwise downregulates CYP7A1.

Finally, *Sandra Schreyer* (Molndahl, Sweden) described the efforts to investigate the role of mitochondrial glycerol-3-phosphate acyl-transferase (mtGPAT) in hepatic triglyceride synthesis. Overexpression of mtGPAT in liver resulted in hepatic steatosis in chow fed mice. However, the mtGPAT knockout mouse showed a surprisingly mild phenotype. When these mice were fed a high fat diet there was no impact on obesity or hepatic triglyceride content suggesting that mtGPAT does not play a critical role in regulating triglyceride synthesis in the liver.

Claudia Coomans (Leiden, The Netherlands) addressed the role of the central nervous system in control of plasma triglyceride metabolism, particularly whether an increase in central insulin availability would influence the tissue-specific distribution of plasma free fatty acids (FFA) and triglyceride-derived fatty acids. It was shown that continuous infusion of small amounts of insulin into the lateral ventricle of fasted mice promotes the uptake of both FFA and triglyceride-bound fatty acids in adipose tissue, but not in liver, heart and muscle. Thus, insulin acts by both direct and indirect (i.e., via the brain) means to stimulate energy storage in adipose tissue.

Peter J Voshol (Leiden, The Netherlands) demonstrated that over-expression of apoC-I aggravates hepatic inflammation, as deduced from increased NF-kB activation, in mice fed a high fat diet. In addition, in spite of the fact that apoC-I overexpression did not alter food intake, body weight gain or energy expenditure of the mice, it did result in a marked peripheral and hepatic insulin resistance, indicative for a modulatory role of apoC-I in the development of several components of the metabolic syndrome.

Leanne Hodson (Oxford, UK) presented results of a stable isotope study in humans addressing the relative contributions of dietary and splanchnic fatty acids to VLDL production by the liver in control and insulin-resistant subjects, the latter with high plasma VLDL-triglyceride levels. She was able to show that, at 6 h after a test meal, most of the difference between the groups was accounted for by a much larger contribution of splanchnic fat in the insulin-resistant subjects: the contribution of dietary fatty acids and endogenous systemic FFA was similar between both groups.

Sabine Rütti (Zürich) studied the effects of HDL isolated from healthy human donors on human and mouse pancreatic β cell turnover and function. Using a variety of in vitro techniques, she was able to show that HDL induces human islet cell proliferation and protects human and mouse islet cells from glucose- and Il-1β-induced apoptosis. Induction of FLIP mRNA expression by HDL was proposed as a potential mechanism. HDL did not affect β-cell secretory function as assessed by glucose-stimulated insulin secretion.

8. Session VI

Varia: chaired by Athina Kalopissis (Paris, France) and Arnold von Eckardstein (Zurich, Switzerland).

Oral contributions at the varia session included, as has become a tradition, a diversity of topics and molecules involved in lipid metabolism and atherosclerosis.

Tim Vanmierlo (Maastricht, The Netherlands) presented new data on Liver X Receptor (LXR) activation in a mouse model of Alzheimer's Disease (AD mice). Because the activation of the LXR pathway results, according to the literature, in

reduced levels of secreted amyloid-β, the cognitive functions of 21 month-old AD mice were evaluated after long-term treatment with the synthetic LXR agonist TO901317. The spatial and object memory of old AD mice was improved, probably through alterations in brain cholesterol metabolism. LXR agonists may provide a future treatment for improvement of clinical outcome of AD patients.

Oliver Bruns (Hamburg, Germany) received this year′s Young Investigator Award for his presentation of "Non-invasive in vivo imaging of recombinant TRL labeled with nanocrystals". To avoid radioactive lipid labels, the lipid core of triglyceride rich lipoproteins (TRL) was labeled with lipophilic superparamagnetic iron oxide nanocrystals. Micelles were formed and apoE and lipoprotein lipase were associated to obtain recombinant TRL (rTRL), which had a diameter of 250 nm. After injection into mice, the turnover and organ uptake of rTRL was followed by dynamic MRI with a time resolution of 3 s. rTRL were rapidly cleared from the blood and taken up into liver (internalized in hepatocytes), spleen and bone marrow. This new labeling technique allows non-invasive imaging to study postprandial lipoprotein kinetics.

Scavenger receptor class B type I (SR-BI) play a major role in selective uptake of HDL-C in rodents. *Menno Vergeer* (Amsterdam, The Netherlands) and colleagues sequenced the SR-BI gene in 162 unrelated subjects with hyperalphalipoproteinemia and described the identification of a subject heterozygous for a point mutation resulting in P297S. Quantitative trait locus (QTL) analyses with 123 members of this subject's family (carriers and unaffected) showed an allelic effect of $+0.39 \pm 0.09$ mmol/L on HDL cholesterol levels. P297S carriers exhibited a 17% increase in apo AI levels, whereas fractional cholesterol efflux from their cultured primary macrophages was 20% lower. This study suggests that SR-BI may be important in controlling HDL metabolism in humans.

The final session of this year's ELC meeting was started by the presentation of the young investigator poster award winner. *Philip Gordts* (Leuven, Belgium) investigated the role of the distal intracellular NPXY-motif of the LDL receptor related protein LRP1. He and his colleagues used a knock-in approach to inactivate the distal NPXY motif of LRP1 in mice. After crossing with LDL-receptor knockout mice these mice showed aggravated dyslipidemia and enhanced atherosclerotic lesion development. Possible reasons include the more atherogenic lipoprotein profile and disturbed PDGF signalling.

Ernst Steyrer (Graz, Austria) investigated the effects of phosphatidylethanolamine *N*-methyltransferase (PEMT) on the formation and stability of lipid droplets. This enzyme converts phosphatidylethanolamine (PE), which has a conical shape, into phosphatidylcholine (PC, cylindrical shape) and is therefore assumed to play an important role in the fission of lipid droplets from the ER. In line with its function, inhibition or absence of PEMT enhanced basal lipolysis in 3T3-L1 adipocytes and in tissue samples from PEMT-knockout mice, respectively. Conversely, overexpression of PEMT decreased free fatty acid release from adipocytes. In summary, lipid droplet stability seems to be affected by the ratio between PE and PC as constituents of the lipid droplet phospholipid monolayer.

By using turnover studies in mice in conjunction with microscopic techniques *Andreas Niemeier* and colleagues (Hamburg, Germany) showed that bone is an important tissue taking up chylomicron remnants from the circulation. On the cellular level, sinusoidal endothelial cells, macrophages and notably osteoblasts were found to bind and internalize chylomicron remnants. Enrichment of chylomicrons with vitamin K led to enhanced carboxylation of osteocalcin in vivo. It is concluded that bone is a physiologically important site of chylomicron remnant uptake, possibly to ensure the supply of bone with lipid soluble vitamins.

The two final presenters exploited the fruit fly Drosophila as a powerful genetic model to study the evolutionary conservation of important players in lipid metabolism and to identify and characterize novel key factors. By using reverse genetics, transcriptomics and subcellular proteomics, *Ronald P. Kühnlein* (Göttingen, Germany) studied fat storage regulation and identified the triglyceride lipase Brummer as a homolog of adipose triglyceride lipase, the lipolytic G-protein coupled receptor AKHR and the PAT domain proteins Lsd-1 and Lsd-2 as relatives of the cage protein perilipin. *Alex P. Gould* (London, UK) identified oenocytes as a cell type which accumulates lipids that are released from fat body during starvation. In addition, oenocytes were found to express more than 20 genes which are involved in lipid metabolism. It is concluded that in insects oenocytes exert lipid processing functions that in mammals are accomplished by the liver.

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For information about the preliminary program and abstract forms, please contact Prof.dr.med. Arnold von Eckardstein, secretary of the ELC (arnold.voneckardstein@IKC.USZ.ch). Updates and forms will be published on the website of the ELC: http://www.elc-tutzing.org.

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